CLAIMS

We claim:

1. A thermostable structure-specific nuclease having an amino acid sequence selected from the group consisting of SEQ ID NOS:102, 107, 130 132, 179, 181, 183, 184, 185, 186, 187, and 188.

2. The nuclease of Claim 1, wherein said nuclease is encoded by a DNA sequence selected from the group consisting of SEQ ID NO:101, 106, 129 131, 178, 180, and 182.

3. A recombinant DNA vector comprising DNA having at least a portion of nucleotide sequence encoding a structure-specific nuclease, wherein said nucleotide sequence is selected from the group consisting of SEQ ID NO:101, 106, 129, 131, 137, 140, 141, 142, 143, 144, 145, 147, 150, 151, 153, 155, 156, 157, 158, 161, 163, 178, 180, and 182.

4. A host cell transformed with the recombinant vector of Claim 3.

5. The host cell of Claim 4, wherein said host cell is an *Escherichia coli* cell.

6. A purified FEN-1 endonuclease selected from the group consisting of *Pyrococcus woesei* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Methanobacterium thermoautotrophicum* FEN-1 endonuclease, *Archaeoglobus fulgidus* FEN-1, and chimerical FEN-1 endonucleases.

7. The purified endonuclease of Claim 6, wherein said endonuclease has a molecular weight of about 38.7 kilodaltons.

8. An isolated oligonucleotide encoding a FEN-1 endonuclease, said oligonucleotide having a region capable of hybridizing an oligonucleotide sequence selected from the group consisting of SEQ ID NOS:108, 109, 112, 113, 116-119, 170, 171, 172, and 173.

9. The isolated oligonucleotide of Claim 8, wherein said oligonucleotide encoding said endonuclease is operably linked to a heterologous promoter.

10. The isolated oligonucleotide of Claim 9, wherein said heterologous promoter is an inducible promoter.

11. The isolated oligonucleotide of Claim 10, wherein said inducible promoter is selected from the group consisting of the λ-P_L promoter, the *tac* promoter, the *trp* promoter and the *trc* promoter.

12. A recombinant DNA vector comprising an isolated oligonucleotide encoding a FEN-1 endonuclease, said oligonucleotide having a region capable of hybridizing an oligonucleotide sequence selected from the group consisting of SEQ ID NOS:108, 109, 112, 113, 116-119, 170. 171, 172, and 173.

13. A host cell transformed with the recombinant vector of Claim 12.

14. The host cell of Claim 13, wherein said host cell is an *Escherichia coli* cell.

15. An isolated oligonucleotide comprising a gene encoding a *Pyrococcus woesei* FEN-1 endonuclease having a molecular weight of about 38.7 kilodaltons.

16. The isolated oligonucleotide of Claim 15, wherein said gene encoding a *Pyrococcus woesei* FEN-1 endonuclease is operably linked to a heterologous promoter.

17. The isolated oligonucleotide of Claim 16, wherein said heterologous promoter is an inducible promoter.

18. The isolated oligonucleotide of Claim 17, wherein said inducible promoter is selected from the group consisting of the λ-P_L promoter, the *tac* promoter, the *trp* promoter and the *trc* promoter.

19. A recombinant DNA vector comprising DNA having a nucleotide sequence encoding a *Pyrococcus woesei* FEN-1 endonuclease having a molecular weight of about 38.7 kilodaltons.

20. A host cell transformed with the recombinant vector of Claim 19.

21. The host cell of Claim 20, wherein said host cell is an *Escherichia coli* cell.

22. A mixture comprising i) a first structure-specific nuclease, wherein said first nuclease consists of a purified FEN-1 endonuclease; and ii) a second structure-specific nuclease.

23. The mixture of Claim 22, wherein said second structure-specific nuclease is selected from the group consisting *Pyrococcus woesei* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1, *Methanococcus jannaschii* FEN-1 endonuclease, *Methanobacterium thermoautotrophicum* FEN-1 endonuclease, *Archaeoglobus fulgidus* FEN-1, and chimerical FEN-1 endonucleases.

24. The mixture of Claim 22, wherein said purified FEN-1 endonuclease is selected from the group consisting *Pyrococcus woesei* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Methanobacterium thermoautotrophicum* FEN-1 endonuclease, *Archaeoglobus fulgidus* FEN-1 endonuclease, and chimerical FEN-1 endonucleases.

25. The mixture of Claim 22, wherein said second nuclease is a 5' nuclease derived from a thermostable DNA polymerase altered in amino acid sequence such that it exhibits reduced DNA synthetic activity from that of the wild-type DNA polymerase but retains substantially the same 5' nuclease activity of the wild-type DNA polymerase.

26. The mixture of Claim 22, wherein said second nuclease is selected from the group consisting of the Cleavase® BN enzyme, *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *Escherichia coli* Exo III, *Saccharomyces cerevisiae* Rad1/Rad10 complex.

27. A method for treating nucleic acid, comprising:

a) providing:

i) a purified FEN-1 endonuclease; and

ii) a nucleic acid substrate;

b) treating said nucleic acid substrate under conditions such that said substrate forms one or more cleavage structures; and

c) reacting said endonuclease with said cleavage structures so that one or more cleavage products are produced.

28. The mixture of Claim 27, wherein said purified FEN-1 endonuclease is selected from the group consisting *Pyrococcus woesei* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Methanobacterium thermoautotrophicum* FEN-1 endonuclease, *Archaeoglobus fulgidus* FEN-1 endonuclease, and chimerical FEN-1 endonucleases.

29. The method of Claim 27, further comprising providing a structure-specific nuclease derived from a thermostable DNA polymerase altered in amino acid sequence such that it exhibits reduced DNA synthetic activity from that of the wild-type DNA polymerase but retains substantially the same 5' nuclease activity of the wild-type DNA polymerase.

30. The method of Claim 29, wherein a portion of the amino acid sequence of said second nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a eubacterial thermophile of the genus *Thermus*.

31. The method of Claim 30, wherein said thermophile is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus* and *Thermus thermophilus*.

32. The method of Claim 31, wherein said structure-specific nuclease is the Cleavase® BN nuclease.

33. The method of Claim 27, wherein said nucleic acid of step (a) is substantially single-stranded.

34. The method of Claim 27, wherein said nucleic acid is selected from the group consisting of RNA and DNA.

35. The method of Claim 27, wherein said nucleic acid of step (a) is double stranded.

36. The method of Claim 35, wherein said treating of step (b) comprises:

i) rendering said double-stranded nucleic acid substantially single-stranded; and

ii) exposing said single-stranded nucleic acid to conditions such that said single-stranded nucleic acid has secondary structure.

37. The method of Claim 36, wherein said double stranded nucleic acid is rendered substantially single-stranded by the use of increased temperature.

38. The method of Claim 37, further comprising the step of detecting said one or more cleavage products.

39. A method for treating nucleic acid, comprising:

a) providing:

i) a first structure-specific nuclease consisting of a purified FEN-1 endonuclease in a solution containing manganese; and

ii) a nucleic acid substrate;

b) treating said nucleic acid substrate with increased temperature such that said substrate is substantially single-stranded;

c) reducing said temperature under conditions such that said single-stranded substrate forms one or more cleavage structures;

d) reacting said cleavage means with said cleavage structures so that one or more cleavage products are produced; and

e) detecting said one or more cleavage products.

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40. The method of Claim 39, wherein said purified FEN-1 endonuclease is selected from the group consisting *Pyrococcus woesei* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Methanobacterium thermoautotrophicum* FEN-1 endonuclease, *Archaeoglobus fulgidus* FEN-1 endonuclease, and chimerical FEN-1 endonucleases.

41. The method of Claim 39, comprising providing a second structure-specific nuclease.

42. The method of Claim 41, wherein said second nuclease is selected from the group consisting of the Cleavase® BN enzyme, *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *Escherichia coli* Exo III, and the *Saccharomyces cerevisiae* Rad1/Rad10 complex.

43. The mixture of Claim 41, wherein said second nuclease is a 5' nuclease derived from a thermostable DNA polymerase altered in amino acid sequence such that it exhibits reduced DNA synthetic activity from that of the wild-type DNA polymerase but retains substantially the same 5' nuclease activity of the wild-type DNA polymerase.

44. The method of Claim 39, wherein said nucleic acid is selected from the group consisting of RNA and DNA.

45. The method of Claim 44, wherein said nucleic acid of step (a) is double stranded.

46. A nucleic acid treatment kit, comprising:

a) a composition comprising purified FEN-1 endonuclease; and

b) a solution containing manganese.

47. The method of Claim 46, wherein said purified FEN-1 endonuclease is selected from the group consisting *Pyrococcus woesei* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Methanobacterium thermoautotrophicum* FEN-1 endonuclease, *Archaeoglobus fulgidus* FEN-1 endonuclease, and chimerical FEN-1 endonucleases.

48. The kit of Claim 46, further comprising a second structure-specific nuclease.

49. The kit of Claim 46, wherein said second nuclease is a 5' nuclease derived from a thermostable DNA polymerase altered in amino acid sequence such that it exhibits reduced DNA synthetic activity from that of the wild-type DNA polymerase but retains substantially the same 5' nuclease activity of the wild-type DNA polymerase.

50. The kit of Claim 48, wherein a portion of the amino acid sequence of said second nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a eubacterial thermophile of the genus *Thermus*.

51. The method of Claim 50, wherein said thermophile is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus* and *Thermus thermophilus*.

52. The kit of Claim 48, further comprising reagents for detecting said cleavage products.